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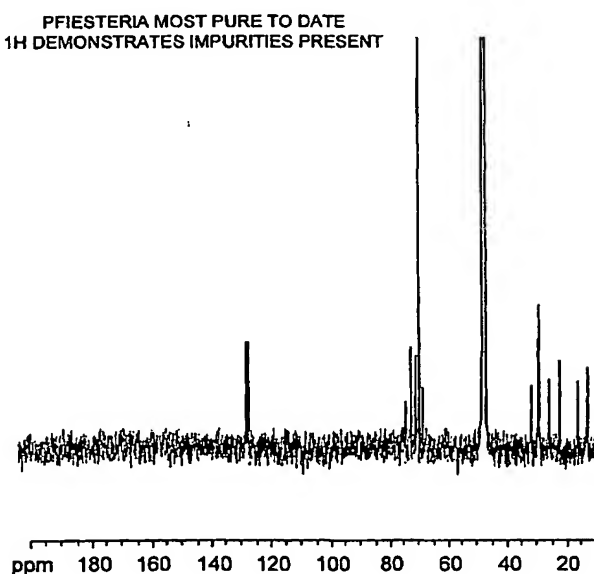
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SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM,  
GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:  
— with international search report

[Continued on next page]

(54) Title: WATER SOLUBLE TOXIN PRODUCED BY PFIESTERIA SPECIES

PFIESTERIA MOST PURE TO DATE  
1H DEMONSTRATES IMPURITIES PRESENT



CURRENT DATA PARAMETERS  
NAME IBF  
EXPNO 2  
PROCNO 1

F2 - ACQUISITION PARAMETERS  
DATA 20010513  
TIME 14.03  
INSTRUM SPECT  
PROBHD 5 mm [X] 13C  
PULPROG zgpg30  
FIDRES 32968  
SOLVENT DMSO  
DSO 27504  
DSC 27777.770 Hz  
SSB 0.847770 Hz  
FIDRES 0.5898740 sec  
AQ 18.6380 usec  
DE 6.00 usec  
TE 300.00 K  
G11 3.00000000 sec  
G11 0.03000000 sec

-----CHANNEL f1-----  
NUC1 13C  
P1 12.80 usec  
PL1 0.00 dB  
SFO1 125.7713156 MHz

-----CHANNEL f2-----  
CPDPRG2 waltz16  
NUC2 1H  
PCPD2 75.00 usec  
PL2 3.00 dB  
PL12 2.00 dB  
SFO2 500.1325000 MHz

F2 - PROCESSING PARAMETERS  
F2 125.7576107 MHz  
WDW EM  
SSB 3.00 Hz  
GB 1.00  
PC

1D NMR PLOT PARAMETERS  
FID 20010513  
F2 125.7576107 MHz  
F3 500.1325000 MHz  
F4 125.7576107 MHz  
F5 125.7576107 MHz  
F6 125.7576107 MHz  
F7 125.7576107 MHz  
F8 125.7576107 MHz  
F9 125.7576107 MHz  
F10 125.7576107 MHz  
F11 125.7576107 MHz  
F12 125.7576107 MHz  
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F96 125.7576107 MHz  
F97 125.7576107 MHz  
F98 125.7576107 MHz  
F99 125.7576107 MHz  
F100 125.7576107 MHz

(57) Abstract: An isolated and purified Pfisteria toxin having the NMR spectra given in Figure 1 herein is described. The toxin may be produced by (a) culturing a Pfisteria species in a growth media to produce Pfisteria toxin therein; (b) separating a first fraction of organic compounds including said Pfisteria toxin from said growth media; and then (c) separating a second fraction consisting essentially of said Pfisteria toxin from said first fraction by chromatography with porous silica beads.



— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(88) Date of publication of the international search report:  
7 October 2004

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/33637

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> IPC(7) : A61K 39/02; C12P 21/04 US CL : 424/236.1; 435/71.3 According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) U.S. : 424/236.1; 435/71.3  Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched National Library of Medicine - MeSH  Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Please See Continuation Sheet		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6,261,848 B1 (ANDERSON et al) 17 July 2001(17.08.2001), column 8, lines 34-47.	5,6,7,12,13,14
A	WO 00/10577 A1 (FUISZ, R.C.) 02 March 2000 (02.03.2000), see pages 20-12.	2,8
A	US 6,391,558 B1 (HENKENS, R.W. et al) 21 May 2002(21.05.2002), column 24, lines 33-44.	2,5,6,8,12,13
A	US 2001/0053556 A1 (ANDERSON, C.W. et al.) 20 December 2001 (20.12.2001), page 5, paragraphs 0072-0073.	5,6,7, 12 ,13 ,14
A	BURKHOLDER, J.M. et al. History of toxic <i>Pfiesteria</i> in North Carolina estuaries form 1991 to the present. Bioscience. October 2001, Vol. 51, No. 10, pages 827-841, page 837, paragraphs 1-2.	2, 8
A	FAIREY, E.R. et al. Reporter gene assay for fish-killing activity produced by <i>Pfiester piscicida</i> . Env. Health Persp. September 1999, Vol. 107, No. 9, pages 711-714.	2,5,6,8,12,13
X	MOELLAR, P.D.R., et al. Current Progress in isolation and characterization of toxins isolated from <i>Pfiesteria piscicida</i> . Env. Health Persp. October 2001, Vol.109(suppl.5), pages 739-743, page 740 Materials and Methods and Fig. 1.	2,5,6,8,12,13
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* Special categories of cited documents:		
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	
"E" earlier application or patent published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family	
"P" document published prior to the international filing date but later than the priority date claimed		
Date of the actual completion of the international search 15 March 2004 (15.03.2004)	Date of mailing of the international search report 18 AUG 2004	
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703)305-3230	Authorized officer Tammy K. Field, Ph.D. Telephone No. (571) 272-0856	

# INTERNATIONAL SEARCH REPORT

PCT/US03/33637

## C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KIMM-BRINSON, K.L. et al. Identification of a P2X7 Receptor in GH4C1 rat pituitary cells: a potential target for a bioactive substance produced by <i>Phiesteria piscicida</i> . <i>Envir. Health Perspect.</i> May 2001, Vol. 5, pages 457-462, pages 457-458, paragraphs 2-6.	2,5,6,8,12,13
Y	GLASGOW, H.B. et al. A second species of ichthyotoxic <i>Pfiesteria</i> ( <i>Vianamoebales</i> , <i>Dinophyceae</i> ) <i>Phycologia</i> . 13 August 2001, Vol. 40, No. 3, pages 234-245, page 235, Materials and Methods, paragraph 2 and page 236, Scanning electron microscopy prep culture.	2,3,5,7,8,12,14
Y	BADEN, D.G. et al. Crystallization and toxicology of T34: a major toxin from Florida's red tide organism ( <i>Ptychodiscus brevis</i> ). <i>Toxicon</i> . 1979, Vol. 19, No. 4, pages 455-462, page 456, Materials and Methods, Purification of T34.	2,8

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/33637

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claim Nos.: 8-13  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:  
Please See Continuation Sheet
3. ☐ Claim Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐  
☐

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

PCT/US03/33637

## Continuation of Box I Reason 2:

There are two claims numbered "8.". For purposes of uninterrupted examination, the second claim 8 will be interpreted as claim 9; claim 9 will be interpreted as claim 10, claim; claim 10 will be interpreted as claim 11; claim 11 will be interpreted as claim 12; claim 12 will be interpreted as claim 13; claim 13 will be interpreted as claim 14 as they read on the method of claim 8 drawn to a method of isolated and purified Pfisteria toxin, comprising the steps of (a), (b), (c).

## Continuation of B. FIELDS SEARCHED Item 3:

Dialog, EAST

Pfisteria, toxin, separation, magnetic, shumwayne, piscicidae, piscicida, piscida